AFM suplimentary notes

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Overview

In order to contribute something to the lab after my 3-month occupancy, I try to organize my experience in using AFM by highlighting some points that are not included in the training book and/or not quite emphasized in the manual and/or really important, so that beginners may learn the basic tricks of using AFM easier by reading this (if they wish and trust this!). Also I have thrown out some defeats of unknown reason so that readers can think of it or get an answer. Each section refers to different part of the procedure, and are arranged in proper order. The section titles are appended by some symbols, indicating which mode(s) are featured in that section:

[t]: tapping mode; [c]: contact mode; [f]: operating in fluid; [a]: operating in air; [all]: all

This notes may not be absolutely correct and accurate but it should be worth for reference!

Note that I have tried contact mode in air and fluid, tapping mode in fluid and force volume mode only. Besides them there are also tapping mode in air, lateral force mode and force modulation mode etc., which I don’t know them in detail yet.

Also readers should at least have read through the training book [1], have an idea about working principle of AFM and maybe Prof. Li has taught you basic technique before reading this. This notes cannot be served as training book.

Fluid cell handling [f]

Before mounting the cantilever substrate into the cell it should be cleaned. The manual [2] suggests that it should be cleaned with a simple bath in a solution of liquid detergent and distilled water. The interior of the cell is gently scrubbed
with antiseptic swab, dried using compressed and filtered air or nitrogen. For me I followed the procedure described by [3], besides that I have not used dish washer. The procedure is as follows: thoroughly clean the cell with dish cleaner, rinse it several times alternately with ethanol and ultra-pure water (which is ready in the lab) and dry it in a nitrogen stream.

The paper also suggested exposing the cell to ultra-violet light to obtain a hydrophilic surface especially near the cantilever chip mounting in order to suppress the formation of air bubbles close to the cantilever (troubles with air-bubbles will be discussed later). I have not tried that before (as I read this near the end of my internship) but I think it is reasonable and worth trying.

Don’t forget to wash and wear the protective skirt properly. Always wear gloves while handling components operated in fluid as oil exists on the surface of the hands.

**Mounting cantilever substrate to tip holder[all]**

This procedure requires careful handling. If you let the cantilever substrate fall on the bench you should observe the cantilever by naked eye or microscope to make sure the cantilever is still attached to the cantilever substrate. Try not to destroy the other side of two cantilevers while mounting. It is easy to identify which side should face up (by microscope or naked eyes), bear this in mind when remounting after dropping.

When mounting cantilever substrate into the fluid cell (tip holder for fluid imaging), try not to scratch the glass window while handling the cantilever substrate, as the glass window should be flat and clean enough to let the laser pass through it without disturbance, which may affect the result. Press the fluid cell harder while it is on the mounting stage if you find there is not enough space for the cantilever substrate to pass through the 'gate'.

After mounting check the cantilever substrate whether it is secured (especially the fluid cell: the cantilever substrate may not be secured after you stop pressing it). Try to align the cantilever substrate better by fine-tuning it by the tweezer after securing.

**Laser alignment [all]**

(This is from the manual [2] but worth being emphasized for new users) If laser’s spot cannot be seen in the laser reflection window, the beam’s location is too far from the cantilever. Just fully tighten both laser aiming screw (the 2 upper screws) (CW) then unscrew (CCW) both screws one full turn.

Place the specimen after you have finished laser alignment as the shadow of the tip on the black stage can help.

Remember the shape of the laser spot reflected from the tips in the laser reflection window. If the laser spot is like rectangular or 'uniform', it is probably
reflected from the cantilever substrate. Always take a look on the stage and see if there is a tip shadow.

A special note concerning this for fluid mode will be discussed later.

**Focusing on surface of glass slide [all]**

While you finish setting up laser alignment and locating tip, you start focusing on the slide surface by bringing the tip to it. Occasionally you will see some spots on focus meanwhile, then move the stage horizontally to check whether the focused spots belong to the slide surface. Usually you can see the image from microscope is much brighter (at illumination around 15 in air/ 20 in fluid) when it is near the surface. The correct surface is brought to focus when he tip and surface is very close.

Do not focus on the lower surface of the slide. You may check it by observing if there is an extra on-focus surface at upper region.

**Deflection signal not updating [all]**

This happens when the tip hits the surface while focusing on surface. After getting rid of the warning window showing low deflection signal, the deflection signal on the screen may stop updating (you know this by turning detector adjustment screws). To solve the problem just press the 'withdraw’ button.

**A full step-by-step preparation of fluid tapping mode [t][f]**

The manual oversimplified this procedure, and as a result I spent about 2 to 3 days to optimize the procedure, and I am confident that this is at least work(I follow it for weeks)!

1. We start from focusing tip. At this stage you don’t have secure the SPM head yet. The tip can be observed through the microscope easier by placing a glass slide on the stage beneath the tip. Remove the glass slide when finish.

2. Pay attention while aligning laser. You can still see the dimmer laser hitting the stage even when the laser is aiming at the cantilever substrate, as the laser reflects serveral times in fluid cell. Move the laser leftward until you see the brighter real laser spot hitting directly to the stage. Then align the laser detector roughly (you will have to do it again later anyway).

3. Now put the specimen on the petri dish. Dry up the back of the specimen a little bit so there is still some moisture, then press it against the petri dish. The specimen should be able to be secured firmly on it. Also put a bare glass slide at the other side of the dish.
4. Then focus the surface of the bare glass slide. Meanwhile pay attention to the petri dish so that it does not hit the optical microscope while focusing. When done, increase the distance between the tip and slide a little bit. Up to now you still don’t have to secure the SPM head.

5. Add fluid on the specimen. The surface tension of the fluid should be able to prevent it from leaking out of the surface (if you don’t add too much). If the fluid leaks, please do it again (remove the specimen, clean the petri dish and repeat step 3) as the specimen is probably no longer secured on the petri dish. The height of the fluid should be roughly high enough to immerse the cantilever. Note that if you are experienced enough, you don’t have to use the bare slide to estimate the position.

6. Now remove the SPM head from the Z-stage groove manually, add little fluid on the fluid cell (the SPM is upside down while doing this) and adjust the position of the petri dish. Use your gloved hand to prevent the excess fluid from dropping from the fluid cell on the stage while putting the SPM head back. Then, as you cannot move the SPM head lower anymore (the laser signal received is too low/void) so you can only add fluid until the fluid can cover the whole lower edge of the protective skirt. Place your dropper around the region of fluid cell region while adding fluid. Try to avoid air bubbles while adding fluid. When you succeed, the image on the screen should be very clear and you can see the cantilever clearly.

7. Sometimes even the fluid covers the whole edge, you still cannot see the clear image, because there is air trapped within the region enclosed by the edge of the skirt, fluid cell and the fluid surface when the edge draws the fluid up. Actually this can be avoided by adding little fluid on the surface of fluid cell in the previous step, as the little fluid can pull up their ‘mates’ from the fluid surface. Anyway it still happens sometimes. You can only do that step again.

8. If you need to start over again, slowly lifting the SPM head out of fluid can avoid leakage of fluid to the dish. Usually after taking the SPM head out the laser spot can be reflected to the detector again (or just a slightly adjustment of laser aiming screw) so you may adjust the height of SPM head. If not and you are in need to adjust the height, rinse the cantilever substrate and fluid cell with fluid or/and resoak it to the fluid on the slide and hope that you don’t have to take out the fluid cell and dry everything.

9. The manual [2] says lowering the cantilever substrate into fluid will cause the laser spot to move towards the fixed end of the cantilever. So you have to readjust the laser aiming by turning the right-rear laser aiming screw very SLIGHTLY CCW to move the laser spot back to free end of the cantilever. Do not move too much, otherwise the laser may hit the cantilever substrate rather than cantilever (esp. when you are using the
short cantilevers, which are best choices for fluid tapping mode). Observing the shape of the spot in the laser reflection window can help you to judge.

10. Air bubbles are due to contamination of the cantilever substrate and the fluid cell, and they can be observed from the screen. You should remove them if they are at/ near the cantilever. The manual [2] suggests: remove the SPM head from the Z-stage groove and rinse the cantilever substrate with fluid. For my experience I succeeded getting rid of bubbles by this method after 7 times of effort. Hope that the UV treatment can really prevent this tragedy. You have to wait a few minutes after rinsing to ensure the bubbles are really gone. It may just be rinsed to other sides but it goes out to the cantilever again later.

11. Now you can at last lock the SPM head and redo the focusing of tips. Bear in mind that when you unlock the SPM head, it lowers a little bit which may destroy the cantilever if the tip is too close to the surface.

12. You should do the tuning of the cantilever in fluid when the distance between the cantilever and sample is \(< 25\mu m\) [2]. To achieve this, make good use of tip up/ tip down control rather than using trackball only. For my experience, the resonance frequency is usually between 30 – 60kHz using short tips, and is different when using another tip. Meanwhile the manual [2] says the frequency is about 8 – 10kHz (but in water). I never try that in water, as I think using my range of frequency can still get good image as long as the peak is sharp enough. I saw there are more peaks in higher frequency area (> 80kHz) but I think it may not be suitable. (just feeling but cannot give an explanation, and I have not tried that for the time being so ... I think we should try!) To determine what peak shapes are suitable you may read [4] for reference.

13. Readjust the laser after tuning as tuning process may change the position of the cantilever.

14. Find your point of interest after tuning as tuning process may change the position of the cantilever.

**Engage [all]**

Engage Failure is often and normal. In some cases engage will succeed if you press engage button again. In contact mode, you may increase the difference between the setpoint and current deflection signal(i.e. larger contact force).

Sometimes unpleasant, high frequency sound (eep) appears while engaging in fluid. (I never hear such sound while engaging in air). The reason for this sound is unknown yet, but this sound can be reduced by decreasing the initial gains. However, for my experience, the sound may be from the unstable specimen such as fluid leakage to the petri dish. I tried to set up the specimen again and no such sound heard.
Wavy line in deflection/amplitude image even at zero scan size [all]

The reason for this is unknown. The wavy line means the cantilever is keeping on oscillating up and down when it stays at one place (zero scan size). It seldom appears when using new tips. The wavy line can be reduced by lowering the gain. It seems does not affect the imaging.

Determining sensitivity [all]

In contact mode, it is always a good practice to switch to force mode after engaging to see if it is a good engage. If you need meaningful deflection value and/or control the force acting on the sample precisely, do the sensitivity determination after every engagement. The photo diode can only give you reading of V but not metric value, and you need to find a hard surface to teach the program (that is determining sensitivity) to calculate the metric value for you. However, if you just need height data, it is not really necessary to know the sensitivity. However you should be aware if the sensitivity is too low (the slope is gentle) if the tip is probing to the hard surface. This may show that the cantilever substrate is not secured or the cantilever has problems. The manual’s instruction on sensitivity is clear enough to understand.

In tapping mode, you don’t even have to switch to force mode before imaging. However if you need meaningful RMS amplitude value, you should do so. But extra caution is needed as it is easier to break the tip/cantilever when operating force mode while tapping.

force curve in contact mode [c]

Remember to adjust the deflection setpoint after engaging by switching to force mode. Find the point of contact by changing the ramp size and start Z-scan. This is well illustrated in the manual.

Bear in mind that there is drifting of the cantilever [2] (mentioned in force mode section). When the tip is far away from the sample, the deflection signal should be in principle uniform regardless the X-Y position and time. But this is not the case. Drifting can be significant when the time duration is large, so if it is important to keep the force acting on the sample constant, you should set zero scan size and observe the force curve occasionally.

Make good use of tip up/tip down function [all]

After checking the force curve (in contact mode) and the scan size is still zero, if the Z centre is near the extreme (extended or retracted) of the Z-piezo range, use tip up/tip down function to bring the Z-centre to about middle.
The maximum range of Z-piezo is 6.67\(\mu m\), the cell sample’s height will often exceed or near this range. So you have to adjust the Z-centre position wisely. For example, while the tip is at glass slide region, adjust the Z-centre position to near the "extended" side, so as to make full use of the Z-piezo range.

The deflection image will be white and dark brown in colour when the Z-centre is at "retracted" and "extended" respectively. There are at least two causes for this: First, the most usual one, the height of the sample exceeds the Z-piezo range and second, contamination of the tip such as sample/debris attaches to the tip. To solve, You may just withdraw the tip and engage it again. You may also try to use tip up/tip down but it does not always work.

If the sample cell is too high or too large, you may consider making mosaic of images (this paper[5] shows an example of mosaic of 7 images of a human aortic endothelial cell) as the edge of the cell is usually easier to be imaged. Also, it is tolerable if just a small region of the cell cannot be imaged in some circumstance. The study [6] pointed out and interpret the artifacts while interpreting the image and is worth for reference.

Scope mode and adjusting gains [all]

If you need to obtain accurate height image, you need to adjust the gains properly indicated in the manual. If the height image is accurate, in scope mode, the trace and retrace line should roughly coincide. If not, the reason may be: the sample is deformed (in contact mode only) and/or false engagement.

Different regions may have their relevant optimum gains. So you have to readjust the gain when you start noticing wavy lines in the image.

Real-time planefit and off-line planefit [all]

In the channel window you can see real-time planefit and off-line planefit. Remember to choose the most suitable choice, as the planefit option can change the data of the image. Study how different choices modify your image.

Scanning speed in tapping mode [t]

One disadvantage of tapping mode is the lower scanning speed. If the speed is too fast, the feedback cannot respond promptly as RMS amplitude is used for modulation (you can imagine that by comparing with deflection modulation), and the result is unclear and low resolution image. However, one paper [7] suggested that phase image works well in high scan rate in aquaous buffer while imaging DNA. It seems that phase lag change is more sensitive than that of RMS amplitude. However phase image needs cautious interpretation. Some researchers tried to interpret the phase lag by various mechanical model and computer simulation [3, 8, 9].
Force volume mode [c]

Before turning to force volume mode you have to make sure that the Z-scan range is large enough if using relative trigger mode (manual has mentioned this). You also have to pick up some points of interest to see if the force curve is satisfactory (to do so, based on my experience, you have to change the scan size to zero first). To reduce the imaging time, minimize the scanning region.

The FV graph shows the deflection of the cantilever at different Z position. However, because of drifting, the FV graph is not really meaningful. Usually the force curves are analysed, then an ‘elasticity’ graph is generated. The followings [10, 11, 12, 13] are different examples of ‘elasticity’ graph.

Note that it is also possible to get FV image in tapping mode, but in author’s knowledge the interpretation of the “force curve” is unknown yet but maybe worth researching.

Patience [all]

Patience is more important than skills. In my experience following every procedure listed by manual cannot guarantee good result, as Prof. Li said, sample quality determines the image quality. However, you will not know if the problems are from the sample unless you try all possible imaging improvement methods.

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References


Samples for atomic force microscopy (AFM) were prepared as follows. Aliquots were removed from NMR samples incubated for 10 days at 10 °C, diluted by a factor of 5 with deionized water and 20 µl was immediately applied to freshly cleaved mica. Research supplementary information. Figure S1 | AFM and EM of protofibrils present in NMR samples. Supplementary Information: Determination of Torsional Spring Constant of AFM Cantilevers: Combining Normal Spring Constant and Classical Beam Theory R. Álvarez-Asencio1), E. Thormann1),a), and M. W. Rutland1),2)b) 1Department of Surface and Corrosion Science, School of Chemical Science and Engineering, KTH, the Royal Institute of Technology, SE-100 44 Stockholm, Sweden. 2Chemistry, Materials and Surfaces, SP Technical Research Institute of Sweden.